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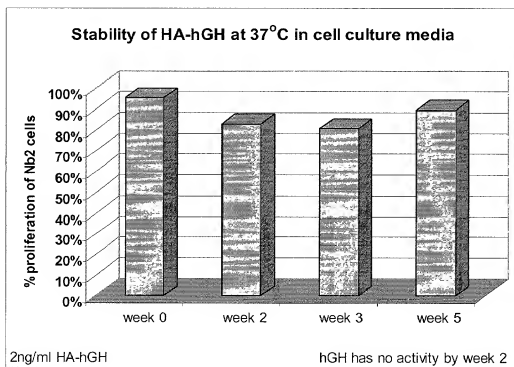


Figure 1

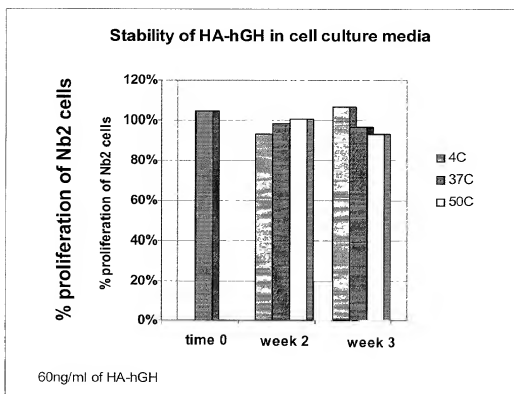


Figure 2

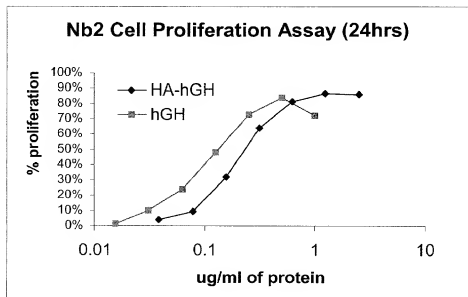


Figure 3A

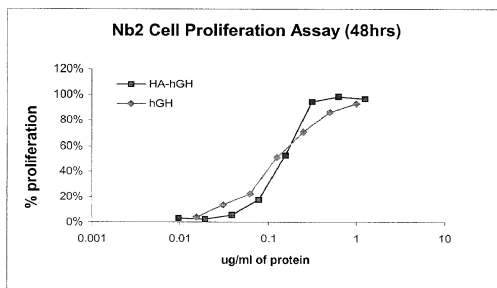


Figure 3B

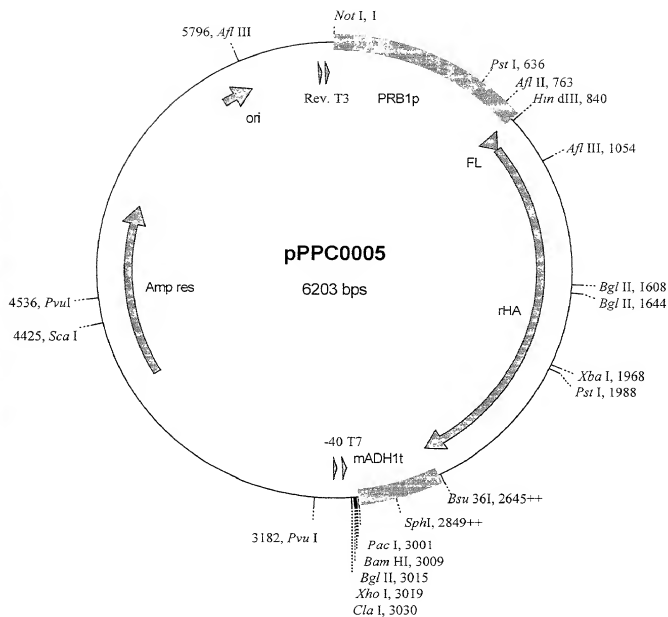


Figure 4

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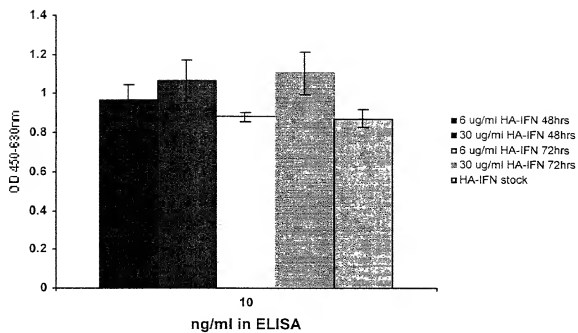


Figure 5

Figure 6

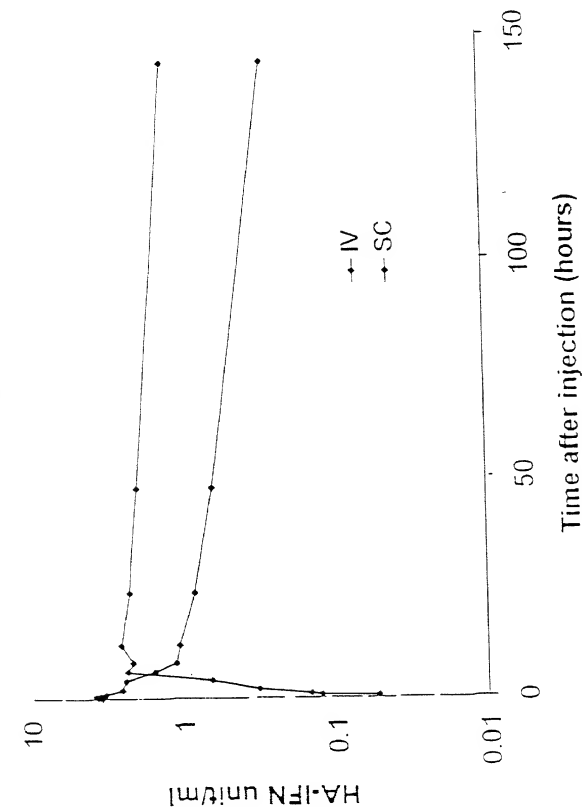
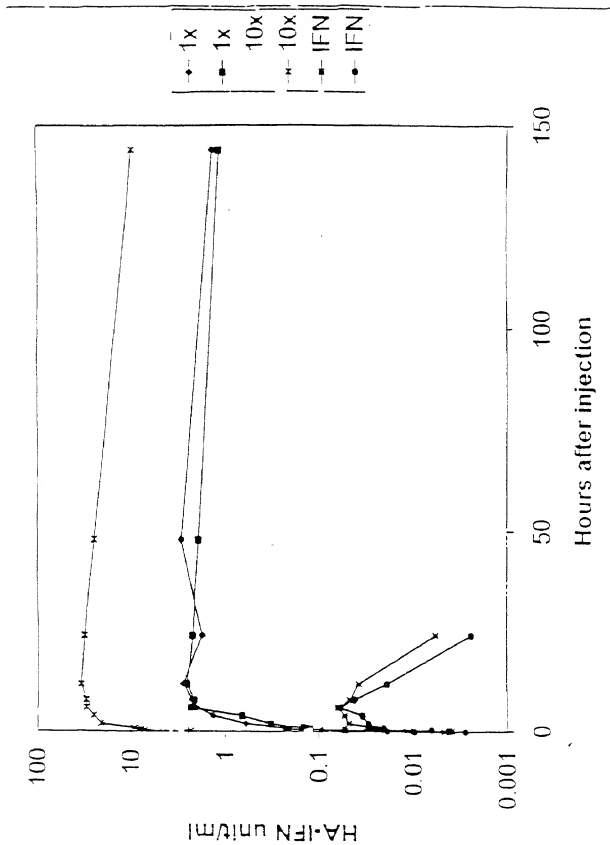


Figure 7



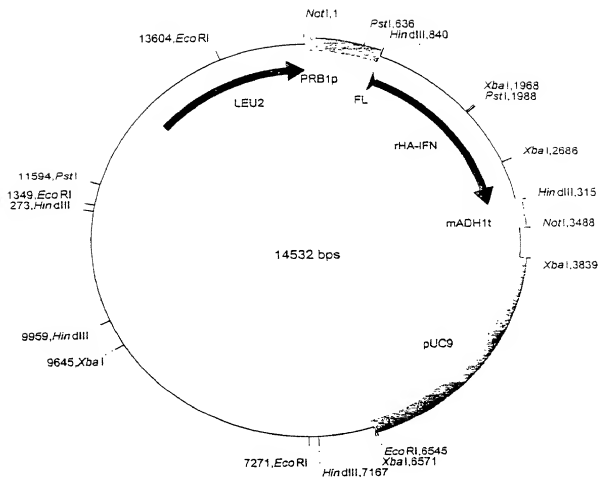


Figure 8. The HA-IFN α expression cassette in pSAC35. The expression cassette comprises

PRB1 promoter, from *S. cerevisiae*.

Fusion leader, first 19 amino acids of the HA leader followed by the last 6 amino acids of the MF α -1 leader.

HA-IFN α coding sequence with a double stop codon (TAATAA)

ADH1 terminator, from *S. cerevisiae*. Modified to remove all the coding sequence normally present in the *Hind* III/*Bam*HI fragment generally used.

Figure 8

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Localisation of 'Loops' based on the HA Crystal Structure
which could be used for Mutation/Insertion

1	DAHKSEVAHR	FKDLGEENFK	ALVLIAFAQY	LQCCPFEDHV	KLVNEVTEFA
	HHHHH	HHH	HHH	HHHHHHHHHH	HHHHH
	I		II		III
51	KTCVADES	AE NCDKSLHTLF	GDKLCTVATL	RBTYGE	MACD CAKOE
	HHHHH	HHHHH	HHHHH	HHHH	H HHHH
101	CFLQHKDDNP	NLPRLVRPEV	DVMCTAFHDN	EETFLKKYLY	BIARRHPYFY
	HHHH	H	HHHHHHHH	HHHHHHHHHH	HHHHH
	IV				
151	APELLFFAKR	YKAAPTECCO	AADKAA	CLLP	KLDEL
	HHHHHHHHHH	HHHHHHHHHH	HHHHH	HHHHHHHHHH	HHHHHHHHHH
	V				
201	ASLQKFGSRA	FKAWAVARLS	QRFPKAEFAE	VSKLVTDLTK	VHTECC
	HHHHH	HH	HHHHHHHHHH	HH	HHH HHHHHHHHHH
	VI		VII		
251	LECADDRADL	AKYICENODS	ISSKLKECCE	KPLLEKSHCI	AEVENDEMPA
	HHHHHHHHHH	HHHHH	HHHHH	HHHHHHH	H
301	DLPSLAADFV	ESKDVCCKNYA	EAKDVFGLGMF	LYEYARRHPD	YSVVLLRLA
	HHHH	HHHHHH	HHHHHHH	HHHHHH	HHHHHHHH
	VIII				
351	KTYETTLKCC	CAAADPHECY	AKVFDEFKPL	VEEPQNLIKQ	NCELFEQLGE
	HHHHHHHHHH	HH	H	HHHHH	HHHHHHHHHH
	IX				
401	YKFQNALVLR	YTKKVPQVST	PTLVEVSRNL	GKVGSKCCKH	PEAKRMPCAE
	HHHHHHHHHH	HHHH	H	HHHHHHHHHH	HHH
	X		XI		
451	DYLSVVLNQL	CVLHEKTPVS	DRVTKCCTES	LVNRRPPCFSA	LEVDETYVPK
	HHHHHHHHHH	HHHHH	HHHHHHHHH	HHHHHHHH	
501	EFNAETFTFH	ADICTLSEKE	RQIKKQATLV	ELVKHKPKAT	KEQLKAVMDD
		HHH	HHH	HHHHHMEHHH	HHH
	XII				
551	FAAFVEKCCK	ADDKETCFAE	EGKKLVAASQ	AALGL	
	HHHHHHHH	HHHH	HHHHHHHHHH	HH	
	Loop		Loop		
	I	Val54-Asn61	VII	Glu280-His288	
	II	Thr76-Asp89	VIII	Ala362-Glu368	
	III	Ala92-Glu100	IX	Lys439-Pro447	
	IV	Gln170-Ala176	X	Val462-Lys475	
	V	His247-Glu252	XI	Thr478-Pro486	
	VI	Glu266-Glu277	XII	Lys560-Thr566	

Figure 9

Examples of Modifications to Loop IV

a. Randomisation of Loop IV.

IV
 151 APELLFFAKR YKAAFTECCQ AADKAACLLP KLDELRLDEGK ASSAKQRLKC
 HHHHHHHHHH HHHHHHHHHH HHHHH HHHHHHHHHHH HHHHHHHHHH

IV
 151 APELLFFAKR YKAAFTECCX XXXXXXCLLP KLDELRLDEGK ASSAKQRLKC
 HHHHHHHHHH HHHHHHHHHH HHHHH HHHHHHHHHHH HHHHHHHHHH

x represents the mutation of the natural amino acid to any other amino acid. One, more or all of the amino acids can be changed in this manner. This figure indicates all the residues have been changed.

b. Insertion (or replacement) of Randomised sequence into Loop IV.

(X)_n

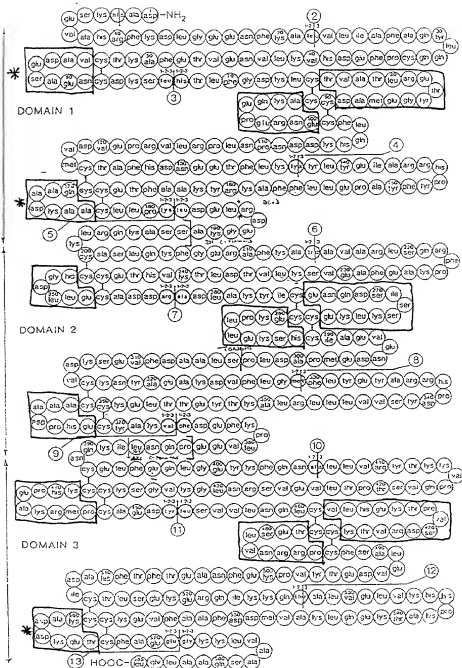


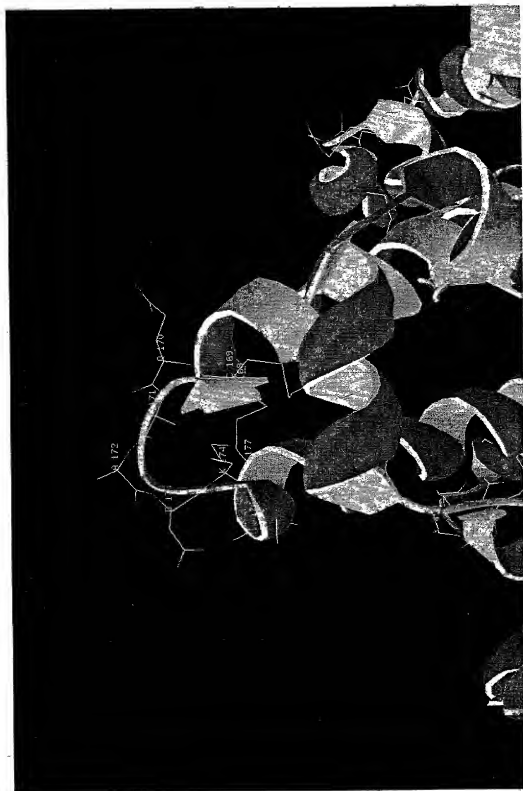
IV

151 APELLFFAKR YKAAFTECCQ AADKAACLLP KLDELRLDEGK ASSAKQRLKC
 HHHHHHHHHH HHHHHHHHHH HHHHH HHHHHHHHHHH HHHHHHHHHH

The insertion can be at any point on the loop and the length a length where n would typically be 6, 8, 12, 20 or 25.

Figure 10





Disulfide bonds shown in yellow

Figure 12: Loop IV Gln170-Ala176



Figure 13: Tertiary Structure of HA

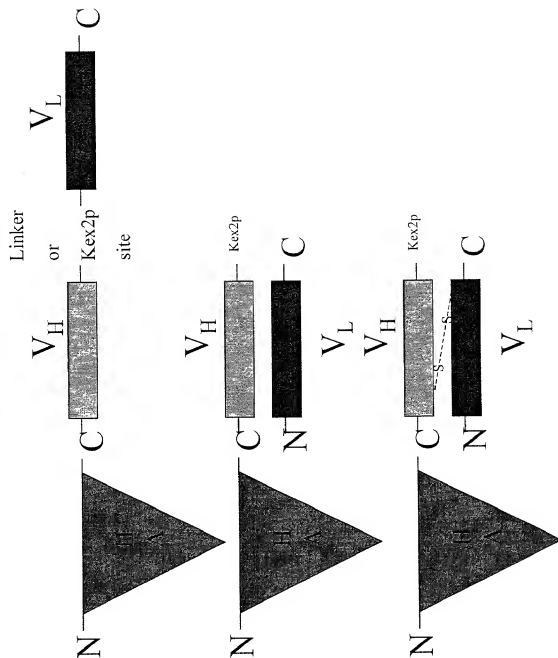


Figure 14: Schematic Diagram of Possible ScFv Fusions
(Example is of a C-terminal fusion to HA)

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1 CAT GCA CAC AAG AGT GAG GTT GCT CGG TTT AAA GAT TTG GGA GAA AAT TTC AAA 60
1 D A H K S E V A H R F K D L G E E N F K 20

61 GCC TTG GTG ATT GCC TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAT CAT GTA 120
21 A L V L I A F A Q Y L Q Q C P F E D H V 40

121 AAA TTA GTG AAT GAA GTA ACT GAA TTT GCA AAA ACA TGT GTT GCT GAT GAG TCA GCT GAA 180
41 K L V N E V T E F A K T C V A D E S A E 60

181 AAT TGT GAC AAA TCA CTT CAT ACC CTT TTT GGA GAC AAA TTA TGC ACA GTT GCA ACT CTT 240
61 N C D K S L H T L F G D K L C T V A T L 80

241 CGT GAA ACC TAT GGT GAA ATG GCT GAC TGC TGT GCA AAA CAA GAA CCT GAG AGA AAT GAA 300
81 R E T Y G E M A D C C A K Q E P E R N E 100

301 TGC TTC TTG CAA CAC AAA GAT GAC AAC CCA AAC CTC CCC CGA TTG GTG AGA CCA GAG GTT 360
101 C F L Q H K D D N P N L P R L V R P E V 120

361 GAT GTG ATG TGC ACT GCT TTT CAT GAC AAT GAA GAG ACA TTT TTG AAA AAA TAC TTA TAT 420
121 D V M C T A F H D N E E T F L K K Y L Y 140

421 GAA ATT GCC AGA AGA CAT CCT TAC TTT TAT GCC CCG GAA CTC CTT TTC TTT GCT AAA AGG 480
141 E I A R R H P Y F Y A P E L L F F A K R 160

Figure 15A

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481 TAT AAA GCT GCT TTT ACA GAA TGT TSC CAA GCT GCT GAT AAA GCT GCC TGC CTG TTG CCA 540
161 Y K A A F T E C C Q A A D K A A C L L P 180

541 AAG CTC GAT GAA CTT CGG GAT GAA GGG AAG GCT TCG TCT GCC AAA CAG AGA CTC AAA TGT 600
181 K L D E L R D E G K A S S A K Q R L K C 200

601 GCC AGT CTC CAA AAA TTT GGA GAA AGA GCT TTC AAA GCA TGG GCA GTG GCT CGC CTG AGC 660
201 A S L Q K F G E R A F K A W A V A R L S 220

661 CAG AGA TTT CCC AAA GCT GAT TTT GCA GAA GTT TCC AAG TTA GTG ACA GAT CTT ACC AAA 720
221 Q R F P K A E F A E V S K L V T D L T K 240

721 GTC CAC ACG GAA TGC TGC CAT GGA GAT CTG CTT GAA TGT GCT GAT GAC AGG GCG GAC CTT 780
241 V H T E C C H G D L L E C A D D R A D L 260

781 GCC AAG TAT ATC TGT GAA AAT CAG GAT TCG ATC TCC AGT AAA CTG AAG GAA TGC TGT GRA 840
261 A K Y I C E N Q D S I S S K L K E C C E 280

841 AAA CCT CTG TTG GAA AAA TCC CAC TGC ATT GCC GAA GTG GAA AAT GAT GAG ATG CCT GCT 900
281 K P L L E K S H C I A E V E N D E M P A 300

901 GAC TTG CCT TCA TTA OCT GCT GAT TTT GTT GAA AGT AAG GAT GTT TGC AAA AAC TAT GCT 960
301 D L P S L A A D F V E S K D V C K N Y A 320

Figure 15B

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961 GAG GCA AAG GAT GTC TTC CTG GGC ATG TTT TTG TAT GAA TAT GCA AGA AGG CAT CCT GAT 1020
 321 E A K D V F L G M F L Y E Y A R R H P D 340

1021 TAC TCT GTC GTG CTG AGA CTT GCC AAG ACA TAT GAA ACC ACT CTA GAG AAG TGC 1080
 341 Y S V V L L L R L A K T Y E T T L E K C 360

1081 TGT GCC GCT GCA GAT CCT CAT GAA TGC TAT GCC AAA GTG TTC GAT GAA TTT AAA CCT CTT 1140
 361 C A A A D P H E C Y A K V F D E F K P L 380

1141 GTG GAA GAG CCT CAG AAT TTA ATC AAA CAA AAC TGT GAG CTT TTT GAG CAG CTT GGA GAG 1200
 381 V E E P Q N L I K Q N C E L F E Q L G E 400

1201 TAC AAA TTC CAG AAT GCG CTA TTA GTT CGT TAC ACC AAG AAA GTA CCC CAA GTG TCA ACT 1260
 401 Y K F Q N A L L V R Y T K K V P Q V S T 420

1261 CCA ACT CTT GTA GAG GTC TCA AGA AAC CTA GGA AAA GTG GGC AGC AAA TGT TGT ABA CAT 1320
 421 P T L V E V S R N L G K V G S K C C K H 440

1321 CCT GAA GCA AAA AGA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC CTG AAC CAG TTA 1380
 441 P E A K R M P C A E D Y L S V V L N Q L 460

1381 TGT GTG TTG CAT GAG AAA CGC CCA GTA AGT GAC AGA GTC ACA AAA TGC TGC ACA GAG TCC 1440
 461 C V L H E K T P V S D R V T K C C T E S 480

Figure 15C

1741 GCT GCC TTA GGC TTA TAA CAT CTA CAT TTA AAA GCA TCT CAG 1782
581 A A I. G L. *

Figure 15D